Interfacial Protein-Lipid Interactions II

2501-Pos

Lipid Targeting of Synaptotagmin I C2 Domains on Asymmetric Two-Phase Planar Bilayers

Chen Wan, Volker Kiessling, Lukas K. Tamm.
University of Virginia, Charlottesville, VA, USA.

We showed previously that cholesterol-rich liquid-ordered domains with lipid compositions typically found in the outer leaflet of plasma membranes could induce liquid-ordered domains in adjacent regions of asymmetric lipid bilayers with apposed leaflets composed of typical inner leaflet lipid mixtures (Biophys.J. 91:3313-26 [2006]). The lipid requirements for this transbilayer coupling in asymmetric cholesterol-rich two-phase lipid bilayers were further investigated and found to be roughly correlated with their chain-melting phase transition temperatures (Biochemistry 47: 2190-8 [2008]). Bilayers containing brain PC (bPC), brain sphingomyelin (bSM) and cholesterol in the outer leaflet and bPC, bPE, POPS and Chol in the inner leaflet, form stable asymmetric two-phase bilayers that are thought to mimic mammalian plasma membranes. In the current work we have studied the calcium-dependent binding and lipid targeting of C2 domains of the presynaptic fusion calcium sensor protein synaptotagmin on asymmetric two-phase bilayers. C2A domains favor disordered over ordered inner leaflet lipid domains. Domain preference does not depend on the PS content in these membranes, but domain preference is more distinct when bPE is left out from the inner leaflet lipid mixture. C2AB tandem domains distinguish less between ordered and disordered lipid domains. Targeting to bilayers containing various amounts of PIP2 in the inner leaflet is also being investigated.

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2502-Pos

Synaptotagmin Perturbs Lipid Structure of Membrane Bilayers

Alex L. Lai, David S. Cafiso.
University of virginia, Charlottesville, VA, USA.

The perturbation of lipid acyl chain order by fusion proteins is widely reported in the membrane of viral entry and fertilization process. Synaptotagmin is the Ca2+ trigger for membrane fusion in neuronal exocytosis, and it may act by modulating lipid packing or membrane curvature strain. The effects of soluble synaptotagmin (C2AB) and the individual C2 domains (C2A and C2B) on the lipid order of POPC:POPS (3:1) membrane bilayer were examined with attenuated total reflection Fourier transformed infrared spectroscopy (ATR-FTIR). Our results show that C2AB and C2B decrease lipid order while C2A increases the lipid order. However, at higher protein concentrations a threshold is reached where, the effect on order is reduced or even reversed. The presence of 1% PIP2 in the lipid bilayer lowers these threshold concentrations. This change in lipid order is largely due to POPS and suggests that the effect on lipid order is due to the demixing of POPS. Interestingly, this effect is not seen in another negatively changed lipid POPG. The effect of synaptotagmin on demixing in PC:PS membrane bilayers was further investigated by fluorescence quenching. Our results show that the C2AB and C2B demix the PS in the presence of Ca2+, while C2A has little effect. Natural abundance 13-C HSQC NMR experiment on POPC:POPS (3:1) vesicles shows that the binding of synaptotagmin changes the chemical shift of PS in the presence of Ca2+, indicating an interaction between the protein and lipid. Taken together, these data suggest that synaptotagmin induces a PS-specific modulation of the acyl chain lipid order as a result of PS-demixing, which may play a role in the mechanism of Ca2+-mediated fusion in the central nervous system.

2503-Pos

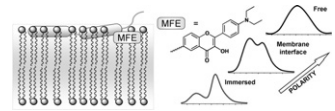
Ratiometric Fluorescent ESIPT Probe Characterizes Binding of Alpha-Synuclein to Membranes

Volodymyr V. Shvadchak, Dmytro A. Yushchenko, Thomas M. Jovin.
MPI for Biophysical Chemistry, Göttingen, Germany.

The aggregation of the protein α-synuclein (AS) is involved in the pathogenesis of Parkinson's disease. Evidence suggests that neurotoxicity may originate from the binding of oligomeric AS to cellular membranes, resulting in disruption and cell leakage. Defining the interactions of AS with membranes is thus essential for understanding its physiological and pathological functions.

For such studies, we developed a cysteine-reactive label (MFE) that senses protein microenvironment via the ratio of two emission bands resulting from Excited State Intramolecular Proton Transfer (ESIPT) [1]. We labeled AS at different positions (ala-to-cysteine mutations) and compared the binding to model membranes and the immersion level of its domains. AS has a greater affinity for membranes with high curvature (SUVs) than to LUVs and for negatively-charged than to neutral membranes. We are currently studying the binding of AS oligomers and the impact of membranes on AS aggregation. For other related studies see refs. [2-4].

[1] Demchenko et al (2009) Biophys J 96: 3461; [2] Celej et al. (2009) Biochemistry 48: 7465; [3] Caarls et al. (2009) J Fluor DOI 10.1007/s10895-009-0536-1; [4] posters by Yushchenko et al. and Fauerbach et al.



2504-Pos

Intermolecular and Intramolecular Interactions and their Role in Lamin a Accumulation at the Nuclear Membrane in Human Aging and Premature Aging Disease

Agnieszka Kalinowski, Siddharth Shenoy, Nikunja Kolluri,
Mathias Loesche, Kris N. Dahl.
Carnegie Mellon University, Pittsburgh, PA, USA.

Hutchison-Gilford progeria syndrome (HGPS) is a premature aging syndrome causing systemic defects. It shows close analogies with normal aging at the molecular and cellular level. It was reported that Δ50 lamin A, the mutant form of an intermediate filament protein in the nucleus, causes lamin accumulation at the nuclear membrane resulting in mechanical anomalies.[1] It is unknown if this membrane accumulation is primarily caused by the deletion of a 50 AA exon or the retention of a post-translational farnesylation on the mutant. We use purified protein fragments, the lamin A tail domain that lacks interactions with other lamins in the structural protein network that supports the nuclear membrane, to quantify changes in protein stability and protein-membrane interactions in the cell and with synthetic membrane models. Over-expressed, labeled Δ50 lamin A tail domains show the same pathologic accumulation at the nuclear membrane as the full-length protein. Circular dichroism indicates no gross structural difference between the *wt* and Δ50 lamin A tail domains, but the unfolding temperature is significantly increased in the latter. This suggests topological differences between the Δ50 lamin A and the *wt* protein which may be responsible for the aging pathology observed *in vivo*. SPR indicates that the binding affinity of the unfarnesylated Δ50 lamin A tail domain to solid-supported, acidic membranes is higher than that of the *wt* lamin A tail domain. This suggests that other consequences of the 50 AA deletion than farnesyl retention may also play a role in the pathological accumulation of the full length protein at the nuclear membrane.

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[1]Dahl, K.N., et al. 2006. PNAS. U.S.A. 103:10271-210276.

2505-Pos

Structure and Cholesterol Binding Properties of the Amyloid Precursor Protein (APP)

Charles R. Sanders[1], Andrew Beel[1], Hak Jun Kim[1], Charles K. Mobley[1], Bing Jap[2], Fang Tian[3], James Prestegard[3], Paul Barrett[1].
[1]Vanderbilt University, Nashville, TN, USA, [2]Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [3]2Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA.

We describe the structure of C99 in model membranes. C99 is the 99-residue transmembrane C-terminal domain of the human amyloid precursor protein (APP), which is liberated by β-secretase cleavage of APP. We also show that cholesterol, an agent that promotes the amyloidogenic pathway associated with Alzheimer's disease, specifically binds to this protein. C99 was purified into model membranes where it was observed to homodimerize. The transmembrane domain of C99 was seen to be an α-helix that is flanked on both sides by mostly-disordered extramembrane domains, with two exceptions. First, there is a short extracellular surface-associated helix located just after the site of non-amyloidogenic α-secretase cleavage that helps to organize the connecting loop to the transmembrane domain, which is known to be essential for amyloid-β production. Second, there is a surface-associated helix located at the cytosolic C-terminus that plays critical roles in APP trafficking and in protein-protein interactions. Cholesterol was seen to associate in a saturable manner with C99, with the binding site being centered at the loop connecting the extracellular helix to the transmembrane domain. Binding of cholesterol to C99/APP may be critical for the trafficking of these proteins to cholesterol-rich membrane domains, which leads to cleavage by β- and γ-secretase and resulting amyloid-β production. These results suggest that APP may serve as a cellular cholesterol sensor that is linked to mechanisms for suppressing cellular cholesterol biosynthesis and uptake. *This work follows up on Beel-AJ et al. (2008) Structural Studies of the Transmembrane C-Terminal Domain of the Amyloid Precursor Protein: Does APP Function as a Cholesterol Sensor? BIOCHEMISTRY, 47, 9428-9446. We thank the*